REMARKS

By this amendment, claim 1 is amended and new claims 43-46 are added.

Claim 1 is amended to make explicit that the nucleic acid directs liver-specific expression of the HCV immunogen. Support for the amendment can be found in the specification, for example, at page 8, lines 2-10; page 13, line 31 through page 32, line 5; and page 14, line 31 through page 15, line 1.

Support for new claims 43-46 can be found in the specification, for example, at page 8, lines 2-10; page 13, line 31 through page 32, line 5; and page 14, line 31 through page 15, line 1.

Thus, claims 1-3, 6, 7, 10-12, 15-21, and 46 are pending in the application. Claims 1-3, 6, 7, 10-12, 15-21, and 41 are under active consideration.

Amendment of the claims is made without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to file one or more continuing applications hereof containing the canceled or unamended claims.

35 U.S.C. § 103

Claims 1-3, 6, 7, 10-12, 15-19, and 41 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over the reference of Gorczynski et al. (Cellular Immunol. (1995) 160:224-231; hereinafter "Gorczynski") in view of Nakai et al. (Blood (1998) 91:4600-4607; hereinafter "Nakai"), and further in view of Wakita et al. (J. Biol. Chem. (1998) 273:9001-9006; hereinafter "Wakita"). Gorczynski is cited for teaching the general concept that animals that are immunologically tolerant to an immunogen can be made by producing the sustained presence of a tolerance inducing immunogen in the liver of an animal. Nakai is cited for teaching a method for sustained expression of a gene in the liver of an animal using an adeno-associated viral particle that expresses human blood coagulation factor IX wherein the adeno-associated viral particle is delivered to the liver by portal vein injection. Wakita is cited for teaching that conditional transgene expression of nucleic acids encoding HCV E1 and HCV E2 in the liver of a trangenic mouse results in an animal that can be used as a tool to investigate the immune responses and pathogenesis of HCV infection.

In addition, claims 1-3, 6, 7, 10-12, 15-21, and 41 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over the reference of Gorczynski et al. (*supra*) in view of Nakai et al. (*supra*), and further in view of Wakita et al. (*supra*) and further in view of Donnelly et al. (WO 97/47358; hereinafter Donnelly). Gorczynski, Nakai and Wakita are applied as above. Donnelly is cited for allegedly teaching that a nucleic acid encoding HCV NS5a could be used to raise an immunological response to HCV in an animal.

In maintaining the rejection, the Advisory Action alleges that it would have been obvious to one of ordinary skill in the art how to create an animal for screening agents that modulate tolerance to HCV:

> It would have been prima facie obvious to one of ordinary skill in the art of creating animal models for screening agents that modulate to a viral immunogen that the cited references could be combined to make the claimed invention with a reasonable expectation of success. Furthermore, one of ordinary skill in the art would understand that making such animal models would be desirable based on the teaching of Wakita that a transgenic mouse that expresses HCV genes in the liver can be used an a model to understand immunological phenomena in HCV infections (as indicated above). It is noted that Applicants have submitted no evidence to support their contention that it is not predictable that tolerance can be achieved for more than one month. Contrary to Applicants' contention, Gorczynski teaches that animals that are immunologically tolerant to an immunogen can be made by producing the sustained presence of a tolerance inducing immunogen in the liver of the animal, and Nakai teaches that a protein of interest can be expressed in the liver of an animal for more than a month. (Advisory Action, page 2.)

Applicant respectfully traverses the rejection and supporting remarks.

There remains a need in the art to find suitable animal models for screening for agents that can modulate or reverse immunological tolerance to HCV antigens. HCV establishes persistent infection in the majority of individuals who become infected in part due to the ability to evade host immune responses by targeting the liver for infection and replication. The instant application describes a method for preparing a non-human animal for screening for agents that modulate tolerance to a hepatitis C virus (HCV) immunogen by exogenously delivering a nucleic acid directing liver-specific expression of an HCV immunogen to the liver of the animal by portal vein injection. None of the references of Gorczynski, Nakai, Wakita, and Donnelly teach or suggest a method for restricting HCV antigens to the liver, as in the instant invention, in order to better mimic the natural development of immunological tolerance to HCV.

Applicant emphasizes that the use of liver-specific promoters and enhancers provides for a superior animal model of HCV tolerance and distinguishes the animal model of the instant invention from those described in the cited references. In support of this position, Applicant submits and relies on a Declaration of Michael Houghton, Ph.D. ("the Declaration"), which addresses the rejection under 35 U.S.C. § 103(a). Dr. Houghton has over 25 years of experience in the field of hepatitis C virology and is the author of numerous publications and patents relating to HCV immunopathology. (See Dr. Houghton's *Curriculum Vitae* attached as Exhibit A).

Dr. Houghton explains in ¶ 7 of the declaration the importance of restricting expression of HCV antigens to the liver in order to mimic the natural biology of HCV:

> HCV replication occurs almost exclusively in the liver where tolerance to the virus develops due to the specialized liver environment, which limits T cell activation and function. See, e.g., Crispe, IN, *Nat. Rev. Immunol.* (2003) 3:51-62, attached as Exhibit B. The use of liver-specific promoters and enhancers in the animal model of the instant invention restricts expression of HCV antigens to the liver, and therefore more accurately mimics the natural development of tolerance to HCV immunogens in the liver during viral infection.

Dr. Houghton points out that the animal model of Wakita does not similarly restrict antigens to the liver:

> Unlike the animal model of the instant invention, expression of HCV antigens in the transgenic animal model described by Wakita is not liver-specific. Genes encoding HCV antigens are present in every tissue of the transgenic mice and are expressed using a CAG promoter that is not liver-specific (page 9002, col. 1). Furthermore, the adenovirus vector encoding the Cre trans gene, which is used to turn on expression of genes encoding HCV antigens, though injected into the tail vein to target the liver, is not restricted to the liver. In fact, expression of HCV antigens is detected in a variety of tissues outside of the liver in Wakita's transgenic mice, including in the lung, spleen, thymus, kidney, stomach, intestines, and muscles (see page 9004, col. 1). Moreover, in the CN2-29 transgenic mice described by Wakita, the expression of the HCV core antigen is at about the same level in the spleen (5.5 ng/mg) as in the liver (6.6 ng/mg) and only about 2-fold less in the lung (2.9 ng/mg). Thus, the transgenic mice of Wakita express HCV antigens in multiple tissues where immunoreactivity is not dampened in the same way as the liver. (Declaration at ¶ 8)

Further, as noted by Dr. Houghton, the animal model of Gorczynski also does not restrict antigens to the liver:

> Similarly, Gorczynski fails to describe or suggest methods for limiting exposure of antigens to the liver of animals. Gorczynski describes injection of lymphoid or spleen cells into the portal vein of mice; however, the cellular antigens are not restricted to the liver and can migrate elsewhere. Thus, both Gorczynski and Wakita describe animal models in which antigens are exposed to the host's immune system outside of the tolerogenic environment of the liver. The instant invention, in contrast, uses liver-specific promoters and enhancers (*e.g.*, alpha-1 anti-trypsin (AAT) promoter and apolipoprotein E (ApoE) enhancer) to ensure that expression of HCV antigens is restricted to the liver, better mimicking the natural biology of the virus and the evolution of tolerance. (Declaration at ¶ 9)

Dr. Houghton also explains that a transgenic mouse, such as described by Wakita, makes a less desirable animal model of immunological tolerance:

> The Wakita transgenic animal model also has a number of additional drawbacks. For one, transgenic animals are usually less desirable as models of tolerance because of the presence of antigens at birth. The immune system views antigens present at birth as "self" antigens and produces long term immunologcal tolerance to self-antigens by thymic deletion of T cells specifically immunoreactive with those antigens. In contrast, the later development of tolerance to non-self antigens by exposure of antigens in the liver has a different underlying mechanism. Therefore, animal models in which antigens are expressed at birth do not provide a good model of tolerance to non-self antigens as develops from exposure of antigens in the liver later in life. See, e.g., Waddington et al., *Curr. Opin. Mol. Ther.* (2007) 9:432-438, attached as Exhibit C. (Declaration at ¶10)

As Dr. Houghton further explains, even though Wakita uses a Cre/loxP system to control expression of HCV antigens, genes encoding the HCV antigens are present in every tissue of the animal and expressed at some level in a variety of tissues outside of the liver:

> Although, Wakita uses the Cre/loxP system for conditional expression of HCV antigens in the transgenic mice, genes encoding the HCV antigens are present at birth in every tissue of the animal and may be expressed at some basal level in the absence of Cre/loxP. For that matter, Wakita observes detectable levels of expression of the HCV core protein in the lung, spleen, thymus, kidney, stomach, intestines, and muscle even though Cre-mediated transgene recombination occurs only in the liver (see page 9004, col. 1). Hence, in these transgenic animals, the immune system may be exposed to HCV antigens due to leaky expression of viral genes, albeit at a low level, from birth. In contrast, the instant application provides a non-germline animal model of tolerance that more accurately mimics the natural development of tolerance during chronic HCV infection. (Declaration at ¶11)

Dr. Houghton further explains other limitations of the animal model of Wakita:

> In addition, it is much more expensive and time consuming to produce numerous transgenic animals, as described by Wakita, for screening for agents that modulate immunological tolerance. The use of portal vein injection of nucleic acids encoding HCV immunogens, as described in the present application, greatly facilitates screening. One of skill in the art can quickly design, for example, a dozen vectors encoding different HCV epitopes and inject such vectors into the portal vein of an animal to test for the development of immunological tolerance and screen for modulators that reverse tolerance. For example, various immunodominant HCV epitopes can be rapidly screened by this method for the development of immunological tolerance and agents that relieve tolerance. Thus, this method greatly increases the ease and flexibility of screening. In contrast, producing a dozen or more transgenic animals, as described by Wakita, to test the same epitopes would entail a great deal more effort, expense, and time. (Declaration at ¶12)

Thus, the animal model of the instant invention presents numerous advantages over those of the prior art for screening for agents that modulate tolerance to HCV immunogens.

Applicant again emphasizes that Gorczynski has nothing to do with methods of preparing an animal model for screening for agents that modulate tolerance to an HCV immunogen. On the contrary, Gorczynski pertains to methods of delaying transplant rejection of skin allografts by injection of lymphoid or spleen cells into the portal vein of an animal. In fact, Gorczynski fails to describe anything pertaining to HCV, and in particular, fails to describe or suggest methods for delivering nucleic acids encoding an HCV immunogen to the liver of an animal or methods for expressing HCV antigens in the liver and sustaining expression in the liver for at least one month in order to induce immunological tolerance. Thus, Gorczynski fails to disclose or suggest the claimed invention.

The secondary reference of Nakai relates to gene therapy and is also not relevant to the presently claimed invention. Nakai pertains to the delivery of a gene encoding human Factor IX for treatment of hemophilia and has nothing to do with immunological tolerance or methods of preparing an animal model for screening for agents that modulate tolerance to an HCV immunogen.

Furthermore, Donnelly has nothing to do with immunological tolerance to HCV. On the contrary, the focus of Donnelly is on therapeutic and prophylactic vaccines capable of eliciting

an immune response against HCV. Thus, Donnelly describes methods for inducing immunity against HCV, which is the opposite of immunological tolerance.

In this case, the combination of Gorczynski, Nakai, Wakita, and Donnelly fails to teach or suggest the method of the claimed invention, including in particular, the injection of a nucleic acid directing liver-specific expression of an HCV immunogen into the portal vein of an animal. Nonetheless, the Examiner has maintained that "it would have been recognized that portal injection of a vector that expresses a protein is an easier way of producing the animal that expresses a foreign gene than making a transgenic animal, as was done by Wakita" (Final Office Action, page 4). However, there is nothing in the prior art as a whole to suggest the desirability of making this combination. The Examiner is using impermissible hindsight reasoning to reconstruct the claimed subject matter by combining references where clearly the advantages of combining the components were unrecognized.

It is axiomatic that statements in the prior art must be considered in the context of the teaching of the entire reference, and that rejection of claims **cannot** be predicated on mere identification in a reference of individual components of claimed limitations. *See, e.g., In re Kotzab* 217 F.3d 1365, 55 USPQ2d 1313, 1317 (CAFC 2000). Thus, the requirement is not whether each claimed element can be identified individually in a reference but, rather, whether the Examiner can show "reasons that the skilled artisan, confronted with the same problem as the inventor, and with no knowledge of the claimed invention, would select the elements from the cited prior art reference for combination in the manner claimed." *In re Rouffet*, 47 USPQ2d at 1458. In the pending case, the Office has not met this burden.

Applicant reminds the Examiner that affidavits by experts can be used to establish what the specification reasonably conveys to the skilled artisan. *In re Alton,* 37 USPQ2d 1578, 1584 (Fed. Cir. 1996). Further, the Patent Office must articulate adequate reasons to rebut a Declaration that properly used facts to arrive at a logically reasoned conclusion (see, *In re Alton, supra*). Thus, if the rejection is maintained, applicant requests clarification regarding the Examiner's position, either in the form of scientific literature, or by a declaration pursuant to 37 CFR §1.104(d)(2). Without such evidence, this rejection must be withdrawn.

For at least these reasons, withdrawal of the rejections under 35 U.S.C. § 103(a) is respectfully requested.

CONCLUSION

In light of the above remarks, Applicant submits that the present application is fully in condition for allowance. Early notice to that effect is earnestly solicited.

If the Examiner contemplates other action, or if a telephone conference would expedite allowance of the claims, Applicant invites the Examiner to contact the undersigned.

The Commissioner is hereby authorized to charge any fees and credit any overpayment of fees which may be required under 37 C.F.R. §1.16, §1.17, or §1.21, to Deposit Account No. 18-1648.

Please direct all further written communications regarding this application to:

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